BROWN & WILLIAMSON TOBACCO CORPORATION

Research Department

Standard Method of Analysis

Method No.: SM-100 Date Issued: July 22, 1982

PROCEDURE FOR THE SIMULTANEOUS DETERMINATION OF NICOTINE AND WATER IN CAMBRIDGE PADS AND NICOTINE IN CIGARETTE FILTER TIPS

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Authors: Mr. M. S. Frank M. S. Frank
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Summary: Cambridge pads or filter tips are extracted with isopropyl alcohol containing two internal standards. Nicotine and water are separated and measured from a single injection using gas chromatography. The injection is split onto separate columns and detectors.

Sample Size: This method is used to measure water and nicotine in samples containing particulate smoke from ten cigarettes or the nicotine in ten cigarette filter tips.

Range and/or Sensitivity: Water can be determined in samples containing from 0.2 to 4.0 mg/cig. (0.1 to 2.0 mg/mL).

Nicotine can be measured routinely from 0.1 to 1.5 mg/cig. (0.05 to 0.75 mg/mL). Higher concentrations can be measured with dilution. (See Appendix III.) Nicotine deliveries as low as 0.001 mg/cig. (0.5 ug/mL) can be measured by reducing the amount of internal standard and increasing sensitivity of the nitrogen - phosphorous detector. (See Appendix III.)

Precision: Water - A precision study was carried out for five weeks. A 1.0 mg/cig. standard solution gave a standard deviation of 0.09 mg/cig. This was equal to a relative standard deviation of 9%. For the cigarette samples, the relative standard deviation ranged from 10% to 60%.

Nicotine - A comparative study between this method and the Auto-Analyzer was carried out along with the water precision study. The precision of this method was equal to that of the AutoAnalyzer. The standard deviation for the 1.0 mg/cig. standard solution was 0.05 mg/cig. The percent relative standard deviation was 5%. For cigarette pads and filters, the relative standard deviation was about 15% or less in most brands.

Analysis Time - Setup: (man-hours) - 6

- Take Down: (man-hours) - 4

- Variable: (man-hours/sample) - 0.1

Approved for Issue: J. A. Tatkow Date: July 23, 1982

F Tall Date: July 23, 1982

1. Principle of Method

Nicotine and water are determined simultaneously using gas chromatography. Cambridge pads and cigarette filters containing whole smoke are extracted with isopropanol which contains two internal standards. The sample is split in a special injection port onto two packed columns. Water, using methanol as the internal standard, is determined using a thermal conductivity detector. Nicotine, with quinoline as the internal standard, is measured using a nitrogen/phosphorous specific detector.

2. Range and Sample Size

2.1 This method can be used to measure water and nicotine in whole smoke from ten cigarettes or nicotine in ten cigarette filters.

2.2 Nicotine can be measured routinely from 0.1 to 1.5 mg/cig. Higher concentrations can be measured with dilution. Nicotine levels as low as 0.001 mg/cig. can be measured with adjustments in the internal standard concentration. (See Appendix III.) The limit of detection is 0.0005 mg/cig. (0.25 µg/mL).

2.3 Water can be measured from 0.2 to 4.0 mg/cig. (0.1 to 2.0 mg/mL).

3. Safety Precautions

Normal safety precautions are employed.

Nicotine is a poison and should be handled with care using rubber gloves in a fume hood.

4. Interferences and Correlative Measurements

None were investigated.

5. Precision and Bias

The method for the simultaneous determination of nicotine and water was highly accurate. This was shown by the small bias of -0.008 mg/cig. observed in a 1.0 mg/cig. water standard solution and the +0.005 mg/cig. bias observed in a 1.0 mg/cig. nicotine standard solution. The percent relative standard deviations (RSD) for the 1.0 mg/cig. water standard and the 1.0 mg/cig. nicotine standard were respectively equal to 9% and 5%.

5.1 Experimental Determination of Precision and Bias

5.1.1 Using the procedure described in this methodology, a precision study for nicotine and water was carried out. At the same time a comparative study (nicotine only) between this method and the AutoAnalyzer method was carried out. For precision estimations, samples from 1.0 mg/cig. standard solutions were analyzed for about five weeks. For the comparison between this method and the AutoAnalyzer, samples of the 1.0 mg/cig. standard solution and samples of cigarette pads and filters were analyzed by both methods for five weeks.

5.1.2 Standard solution samples of nicotine and water were analyzed between cigarette samples. The same cigarette samples were analyzed by both methods.

5.1.3 BCR-2 (BARCLAY) and KSL-3 (KOOL) were used as cigarette control samples. Several samples of each were analyzed daily.

5.1.4 The method was calibrated as outlined in Section 8. The calibration graphs for nicotine and water were linear over the range of 0.1 to 1.5 mg/cig. for nicotine and 0.25 to 4.0 mg/cig. for water.

5.2 Method Bias

5.2.1 The nicotine and water bias of this method was assessed using data from 1.0 mg/cig. standard solutions which were analyzed between cigarette samples.

5.2.2 The observed mean value for this 1.0 mg/cig. nicotine standard was 1.006 mg/cig.; therefore, the bias was +0.006 mg/cig. However, statistical testing showed that the bias was not significantly different from zero.

5.2.3 The observed mean value for the 1.0 mg/cig. water standard solution was 0.992 mg/cig.; hence, the bias was -0.008 mg/cig. Again, the statistical testing showed that the bias was not significantly different from zero.

5.2.4 For pad nicotine results, the differences between this method and the AutoAnalyzer were small. On average, this method gave results which were 4% lower than those found by the AutoAnalyzer. For filter nicotine results, the differences between these methods depended on the cigarette brand tested. The differences for a number of brands are shown in the table on page 7 and 8. A more complete listing of differences for the brands is given in Appendix IV.

5.3 Method Precision

5.3.1 The estimated standard deviation for the 1.0 nicotine standard solution was 0.05 mg/cig. The relative standard deviation was 5%. For cigarette pad and filter samples, the standard deviation at the 0.2 mg/cig. nicotine level was about 0.08 mg/cig. and at the 1.0 mg/cig. level was about 0.12 mg/cig. The relative standard deviations for most of the cigarette brands were less than 15% in pad and filter nicotine analyses.

Similar magnitudes in standard deviations were observed in the AutoAnalyzer method which analyzed the same cigarette samples.

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DIFFERENCE IN NICOTINE RESULTS BETWEEN THE AUTOANALYZER AND GAS CHROMATOGRAPHIC METHODS AND THE PRECISION OF EACH METHOD

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BRAND DESCRIPTION		PAD ANALYSIS NUMBER OF RESULTS	PAD ANALYSIS MEAN RESULT	PAD ANALYSIS STANDARD DEVIATION	FILTER ANALYSIS NUMBER OF RESULTS	FILTER ANALYSIS MEAN RESULT	FILTER ANALYSIS STANDARD DEVIATION	NRE NUMBER OF RESULTS	NRE MEAN RESULT	NRE STANDARD DEVIATION
BARCLAY BOX, PETERSBURG										
	BY AA	242	0.392	0.061	167	0.465	0.092	161	73.04	3.68
	BY GC	242	0.203	0.077	167	0.531	0.129	161	74.81	4.68
	MEAN DIFFERENCE	242	-0.012*	0.031	167	-0.066*	0.061	161	-1.76	2.70
BARCLAY MENTHOL 100, LOUISVILLE										
	BY AA	115	0.322	0.083	97	0.339	0.043	97	52.44	4.44
	BY GC	115	0.325	0.089	97	0.356	0.049	97	53.65	5.33
	MEAN DIFFERENCE	115	-0.003	0.028	97	-0.016*	0.033	97	-1.21	2.42
BARCLAY MENTHOL 100, PETERSBURG										
	BY AA	103	0.373	0.069	34	0.412	0.046	33	51.75	4.30
	BY GC	103	0.417	0.077	34	0.454	0.050	33	51.32	4.57
	MEAN DIFFERENCE	103	-0.044*	0.030	34	-0.042*	0.028	33	0.43	2.14
BARCLAY MENTHOL, LOUISVILLE										
	BY AA	83	0.132	0.074	72	0.288	0.044	70	69.87	4.79
	BY GC	83	0.114	0.070	72	0.312	0.046	70	74.38	4.45
	MEAN DIFFERENCE	83	0.017*	0.016	72	-0.024*	0.026	70	-4.51	2.45
BARCLAY MENTHOL, PETERSBURG										
	BY AA	94	0.187	0.046	39	0.384	0.066	38	69.04	3.80
	BY GC	94	0.180	0.050	39	0.386	0.052	38	70.28	3.51
	MEAN DIFFERENCE	94	0.007*	0.028	39	-0.002	0.040	38	-1.25	3.76
BARCLAY 100, LOUISVILLE										
	BY AA	179	0.344	0.072	132	0.355	0.041	127	51.03	3.33
	BY GC	179	0.335	0.043	132	0.358	0.043	127	52.31	3.93
	MEAN DIFFERENCE	179	0.009*	0.053	132	-0.003	0.039	127	-1.27	2.77
BARCLAY, LOUISVILLE										
	BY AA	122	0.161	0.055	89	0.410	0.083	85	72.24	4.27
	BY GC	122	0.141	0.053	89	0.459	0.084	85	76.84	4.37
	MEAN DIFFERENCE	122	0.020*	0.026	89	-0.049*	0.042	85	-4.60	2.61
BARCLAY, PETERSBURG										
	BY AA	231	0.175	0.052	140	0.426	0.074	133	72.61	4.20
	BY GC	231	0.177	0.063	140	0.480	0.101	133	75.20	4.61
	MEAN DIFFERENCE	231	-0.002	0.029	140	-0.055*	0.053	133	-2.59	2.80
BCP - 2										
	BY AA	369	0.253	0.077	298	0.591	0.073	280	70.87	4.32
	BY GC	369	0.245	0.065	298	0.643	0.081	280	73.16	4.31
	MEAN DIFFERENCE	369	0.008*	0.043	298	-0.052*	0.065	280	-2.28	3.09
BELAIRE 100, PETERSBURG										
	BY AA	39	0.840	0.127	33	0.575	0.045	33	40.79	3.29
	BY GC	39	0.825	0.142	33	0.510	0.042	33	38.50	3.51
	MEAN DIFFERENCE	39	0.015	0.045	33	0.064*	0.031	33	2.29	1.68
KENT GOLDEN LIGHTS KS, PETERSBURG										
	BY AA	41	0.555	0.084	34	0.502	0.048	34	47.55	2.51
	BY GC	41	0.540	0.096	34	0.483	0.049	34	47.76	3.04
	MEAN DIFFERENCE	41	0.015*	0.037	34	0.019*	0.026	34	-0.18	1.42
KOOL BOX 4MIT, PETERSBURG										
	BY AA	99	1.204	0.166	72	0.737	0.092	71	39.03	5.77
	BY GC	99	1.155	0.190	72	0.606	0.076	71	35.82	6.49
	MEAN DIFFERENCE	99	0.054*	0.054	72	0.131*	0.069	71	3.21	2.57
KOOL KING 85ET, MACON										
	BY AA	80	1.351	0.202	61	0.675	0.092	61	37.99	4.54
	BY GC	80	1.109	0.196	61	0.536	0.074	61	33.69	5.20
	MEAN DIFFERENCE	80	0.042*	0.047	61	0.138*	0.050	61	4.30	1.90

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KOOL LIGHT 100 95M1, MACON									
BY AA	72	0.895	0.088	60	0.716	0.047	60	44.37	2.17
BY GC	72	0.921	0.090	60	0.666	0.062	60	41.87	2.03
MEAN DIFFERENCE	72	-0.026*	0.083	60	0.050*	0.054	60	2.50	2.26
KOOL LIGHT 100, PETERSBURG									
BY AA	96	0.812	0.123	77	0.669	0.074	74	45.30	3.64
BY GC	96	0.796	0.127	77	0.638	0.067	74	44.88	3.39
MEAN DIFFERENCE	96	0.015*	0.066	77	0.031*	0.048	74	0.42	2.34
KOOL LIGHT, PETERSBURG									
BY AA	31	0.723	0.083						
BY GC	31	0.711	0.074						
MEAN DIFFERENCE	31	0.012	0.038						
KOOL MILD 100, LOUISVILLE									
BY AA	63	0.904	0.100	43	0.630	0.076	40	41.30	2.75
BY GC	63	0.917	0.130	43	0.547	0.056	40	38.72	3.10
MEAN DIFFERENCE	63	-0.013	0.085	43	0.083*	0.043	40	2.59	1.39
KOOL MILD, MACON									
BY AA	38	0.831	0.133	34	0.768	0.085			
BY GC	38	0.812	0.120	34	0.672	0.086			
MEAN DIFFERENCE	38	0.018	0.070	34	0.096*	0.063			
KOOL ULTRA 100, LOUISVILLE									
BY AA	74	0.491	0.071	47	0.443	0.048	38	46.24	3.09
BY GC	74	0.539	0.089	47	0.463	0.061	38	45.38	3.57
MEAN DIFFERENCE	74	-0.048*	0.062	47	-0.020*	0.041	38	0.87	1.90
KOOL ULTRA, LOUISVILLE									
BY AA	68	0.208	0.046	35	0.343	0.049	30	62.93	2.89
BY GC	68	0.215	0.069	35	0.374	0.061	30	65.08	4.28
MEAN DIFFERENCE	68	-0.007	0.034	35	-0.031*	0.037	30	-2.16	2.56
KSL - 3									
BY AA	395	0.566	0.075	283	0.644	0.057	272	53.84	3.08
BY GC	395	0.562	0.083	283	0.57*	0.071	272	51.5*	4.4*
MEAN DIFFERENCE	395	-0.002	0.066	283	0.065*	0.063	272	2.25	3.76
VICEROY RICH LIGHTS 100, PETERSBURG									
BY AA	30	0.801	0.155	30	0.755	0.110	30	48.75	4.48
BY GC	30	0.787	0.144	30	0.69[illegible]	0.110	30	47.14	4.63
MEAN DIFFERENCE	30	0.014	0.055	30	0.057*	0.045	30	1.60	1.73
VICEROY RICH LIGHTS, JAPAN									
BY AA	36	1.012	0.085						
BY GC	36	0.974	0.093						
MEAN DIFFERENCE	36	0.038*	0.056						

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5.3.2 The estimated standard deviation for the 1.0 mg/cig. water standard was 0.09 mg/cig. The relative standard deviation was 9%. For cigarette samples, the standard deviations ranged from 0.1 mg/cig. to 0.7 mg/cig. as the water levels ranged from 0.2 mg/cig. to 2.3 mg/cig.

5.3.3 For BCR-2 cigarette samples, the estimated within-day standard deviations were 0.06 and 0.08 mg/cig. respectively, for pad and filter. For KSL-3 samples, the estimated within-day standard deviations were 0.08 and 0.07 mg/cig. respectively for pad and filter.

For water samples from the BCR-2 and KSL-3, the estimated within-day standard deviations were 0.19 and 0.18 mg/cig. for BCR-2 and KSL-3 respectively.

6. Apparatus

6.1 Hewlett-Packard 5730 Gas Chromatograph or equivalent, equipped with a nitrogen/phosphorous specific detector and a thermal conductivity detector.

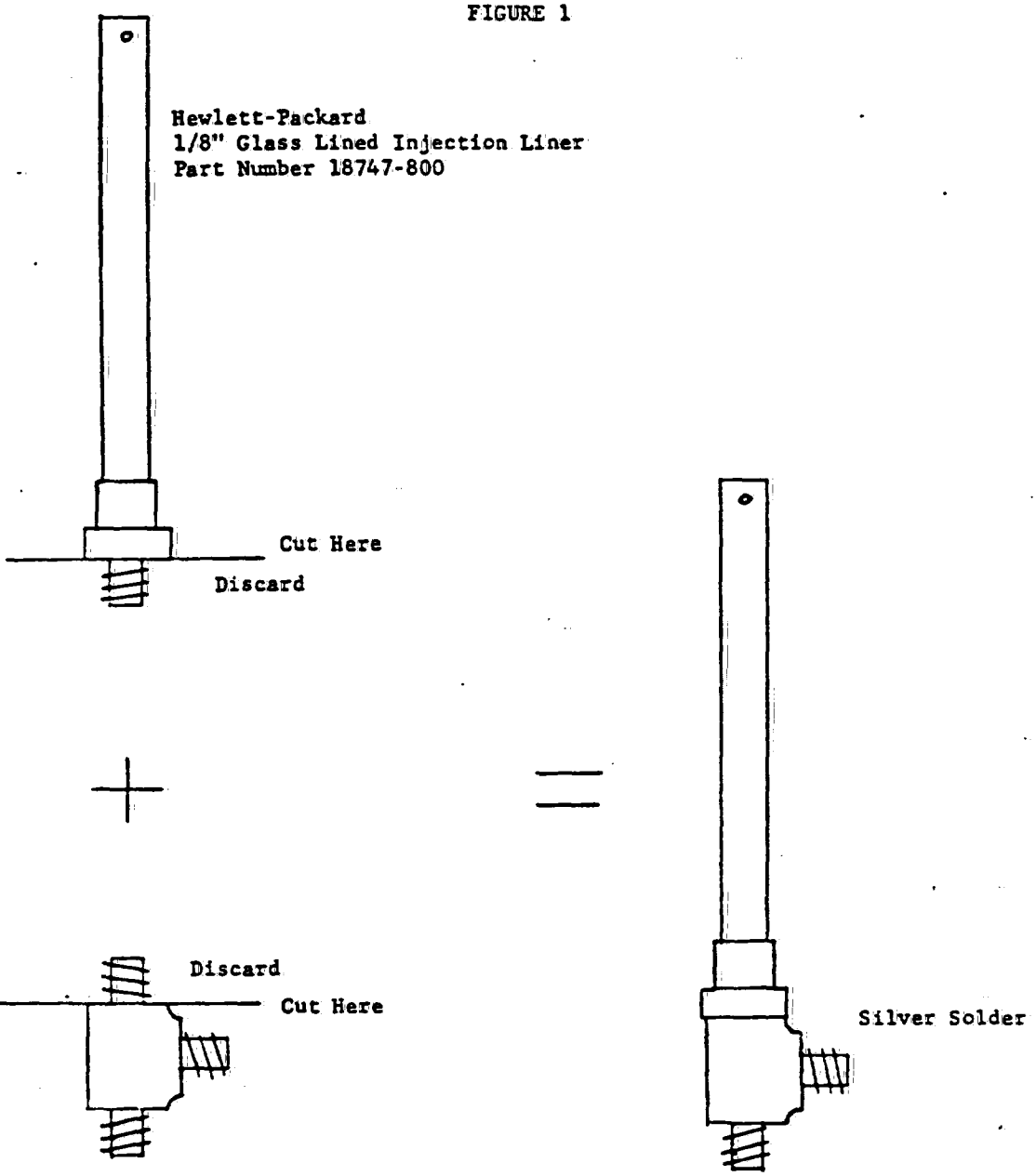
6.2 Hewlett-Packard 7672A auto sampler, or equivalent, equipped to hold 99 samples.

6.3 Column for nicotine - 4' x 1/8" stainless steel packed with 10% Carbowax 20M + 2% KOH on 80/100 mesh Chromosorb W AW. Use Graphlok ferrules. Available from Alltech, Inc. (preconditioned).

6.4 Column for water - 8' x 1/8" copper packed with 50/80 mesh Porapak Q. Use Graphlok ferrules. Available from Alltech, Inc. (preconditioned).

6.5 Modified injection port liner for "A" injector. This was fabricated in the B&W Machine Shop. (See Figure 1.)

FIGURE 1



Swagelok-1/8" Stainless Steel Tee
Part Number 200-3-316

Modified Injection Port Liner

6.6 Column ferrules - 1/8" Graphloks (one piece) from Supelco, Inc., Catalog #2-2603.

6.7 Injection port liner ferrules - 1/4" Graphloks (one piece) from Supelco, Inc. Catalog #2-2604.

6.8 "Up and Down" sample shaker - fabricated in B&W Machine Shop from a Frigidare washing machine mechanism.

6.9 Extraction vials with caps - 29 mm O.D. x 65 mm long, 30 mL capacity from Preiser, Inc., Catalog #10-5032-25.

6.10 Auto sampler sample vials - 1 mL capacity by Wheaton from Preiser, Inc., Catalog #10-4788-12.

6.11 Auto sampler sample vial caps - 11 mm O.D. from Preiser, Inc., Catalog #10-4805-29.

6.12 Auto sampler sample vial cap crimper by Wheaton from Preiser, Inc., Catalog #10-4814-13.

6.13 Labindustries Micro Pipettors are used to make standard solutions.

Catalog No.	Volume (µl)
MP-5H	5
MP-10H	10
MP-20H	20
MP-30H	30
MP-40H (Special Order)	40
MP-20H	20
MP-100H	100
MP-200H	200

Labindustries
620 Hearst Avenue
Berkeley, CA 94710

7. Reagents and Standards

7.1 The following reagents are required:

(a) Nicotine (distilled) GLC assay 98%, Eastman Catalog #1242.

(b) Quinoline - ACS Reagent Grade - GLC assay 99%, Eastman Catalog #218 or Alfa Products, Catalog #11927.

(c) Methanol - ACS Reagent Grade - GLC assay 99%, Eastman Catalog #13032.

(d) Isopropanol - ASC Reagent Grade - Spectro Grade, Water - 0.2% max.

(e) Water - Reagent Grade from Millipore Reverse Osmosis unit.

7.2 Extracting Solution

Unopened five-gallon cans of isopropanol are to be used. Under normal smoking conditions, i.e., eight machine loads/day, this will last two working days. The unused extracting solution should be discarded at the end of the second working day.

7.2.1 Pipette 25 mL of quinoline and 70 mL of methanol into the can of isopropanol after opening. Stopper and mix well.

Label: Internal Standard Added
Date -
Technician -

7.2.2 ISOPROPANOL PICKS UP WATER EXTREMELY FAST!
KEEP THE ISOPROPANOL STOPPERED AT ALL TIMES.

7.2.3 Methanol is the water internal standard and quinoline is the nicotine internal standard.

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7.3 Combined Nicotine/Water Standards

These standards must be made in glassware, extraction vials, transfer syringes, etc., that have been oven dried at 125°C for at least four hours and cooled in a dessicator over Dryrite. The standards must be made, transfered, and capped in a "dry-box" under anhydrous conditions.

Nicotine used to make the standards must be redistilled under reduced pressure in a nitrogen atmosphere. The nicotine must be colorless. Nicotine stored in the dark at -10°C to -20°C under nitrogen will remain colorless for about six months. See Appendix I for nicotine distillation procedure.

Water used to make standards must be fresh distilled water.

7.3.1 Nicotine Stock Solution

Weigh exactly 500 mg (0.500 g) of redistilled nicotine into a 10 mL volumetric flask and dilute to volume with isopropanol that contains no internal standard. After thorough mixing, transfer the solution to a glass stoppered 10 mL Erlenmeyer flask. Concentration = 50 mg/mL or 0.5 mg/10 µL.

Label: Nicotine Stock Solution
0.5 mg/10 µL
Date _
Technician Name -

7.3.2 Nicotine/Water Working Standards

Place two whole Cambridge pads and two quarter pads into each of seven extraction vials. Add water and nicotine stock solution as shown in Table 1 to these vials using Labindustries micropipettors. The zero water addition level is the solvent blank. After the correct amount of water and nicotine stock solution have been added to each vial, 20 mL of extracting solution is added by pipette or autopipette and the vials shaken on an "up and down" shaker for 15 minutes.

Label: Nicotine/Water Working Standard
Concentration -
Date -
Technician -

7.3.3 Return extracted standards to the "dry-box" and carefully transfer aliquots to the sample vials. Cap the sample vials. Use a clean, dry syringe to transfer each standard.

7.3.4 All standards except the 0.25 mg/cig. nicotine are made by pipetting 20 mL of extracting solution to the stock solutions. The 0.25 mg/cig. nicotine standard is made by pipetting 40 mL of extracting solution to the stock solutions.

TABLE 1

COMBINED NICOTINE/WATER STANDARDS

Standards

Micropipettor Volume (µL)			Concentration (mg/cig.)*	
Nicotine Stock	Distilled Water		Nicotine	Water
20	0	Cal. Std.**	0.1	Blank
300	30	Cal. Std.**	1.5	3.0
200	10	Control	1.0	1.0
100	5		0.5	0.5
0	20		0	2.0
0	40		0	4.0
0	5	(40 mL Ex. Sol)	0	0.25

*These concentrations are based on the assumption that five cigarettes are smoked per pad and two pads are extracted with 20 mL of extracting solution.

**Calibration standards for PAMILA computer system.

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7.4 For best results and ease of sample handling, make a set of standards for each GC to be standardized.

7.5 Approximately three auto sampler vials (1.5 mL capacity) can be filled from a working standard extract.

7.6 Each standard vial can be used for only one injection because water is picked up through the needle puncture.

7.7 The accuracy of standards made using the Labindustries "push button" pipettors should be checked at regular intervals against standards made using Class A volumetric pipettes.

8. Standardization or Calibration

8.1 Set up the instrumentation as described in Section 9.2

8.2 Make five replicate injections of a smoke sample to condition the column system.

8.3 Using a clean, dry 10 µL syringe, make duplicate 2 µL injections of the standards in order.

8.4 Compounds elute from the water column as follows:

Compound	Time (min.)
Air	0.20
Water	0.30
Methanol (Internal Standard)	0.40
Isopropanol (Solvent)	1.00

See Figure 2 for a typical chromatogram of a standard (water).

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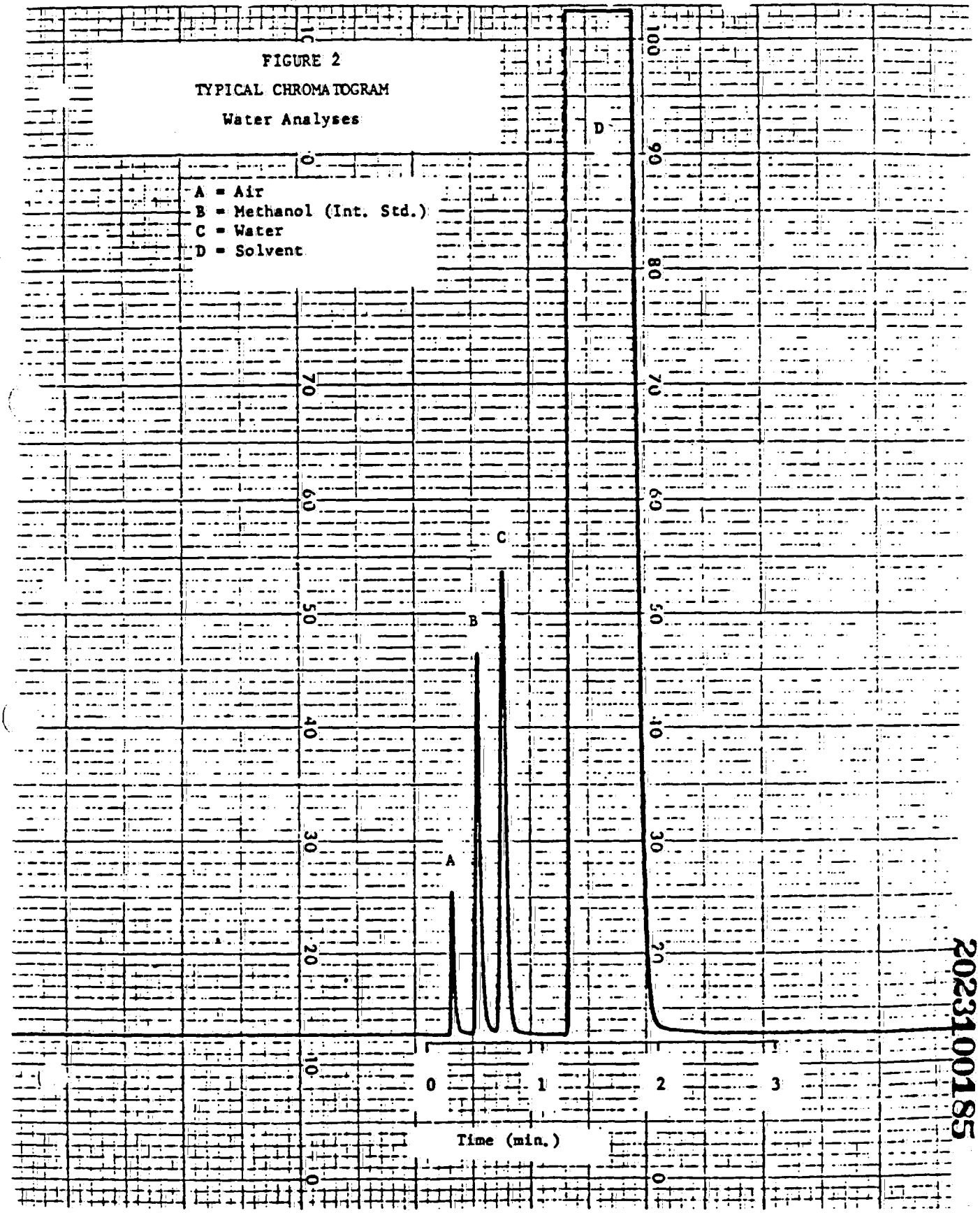
FIGURE 2

TYPICAL CHROMATOGRAM

Water Analyses

A = Air
B = Methanol (Int. Std.)
C = Water
D = Solvent



8.5 Compounds elute from the nicotine column as follows:

Compound	Time (min.)
Isopropanol (Solvent)	0.20
Nicotine	1.40
Quinoline (Internal Standard)	2.00

See Figure 3 for a typical chromatogram of a standard (nicotine).

8.6 This methodology assumes that peak areas will be determined by an integrator or computer. However, peak height measurements can be used for manual calculations.

8.7 Determine the peak area ratios for both the nicotine and the water standards as follows:

$$\frac{\text{Peak area (water or nicotine)}}{\text{Peak area internal standard}} = \text{Peak Area Ratio}$$

8.8 Using the method of least squares, carry out linear regression analysis and determine the slope, Y-intercept, and correlation coefficient.

8.9 The correlation coefficient must be greater than 0.98 or the standardization must be repeated.

8.10 Substitute the slope and Y-intercept in the following linear regression equation:

$$X = \frac{Y - (\text{Y-intercept})}{\text{Slope}}$$

X = amount of water or nicotine in mg/cig.

Y = peak area ratio of water or nicotine

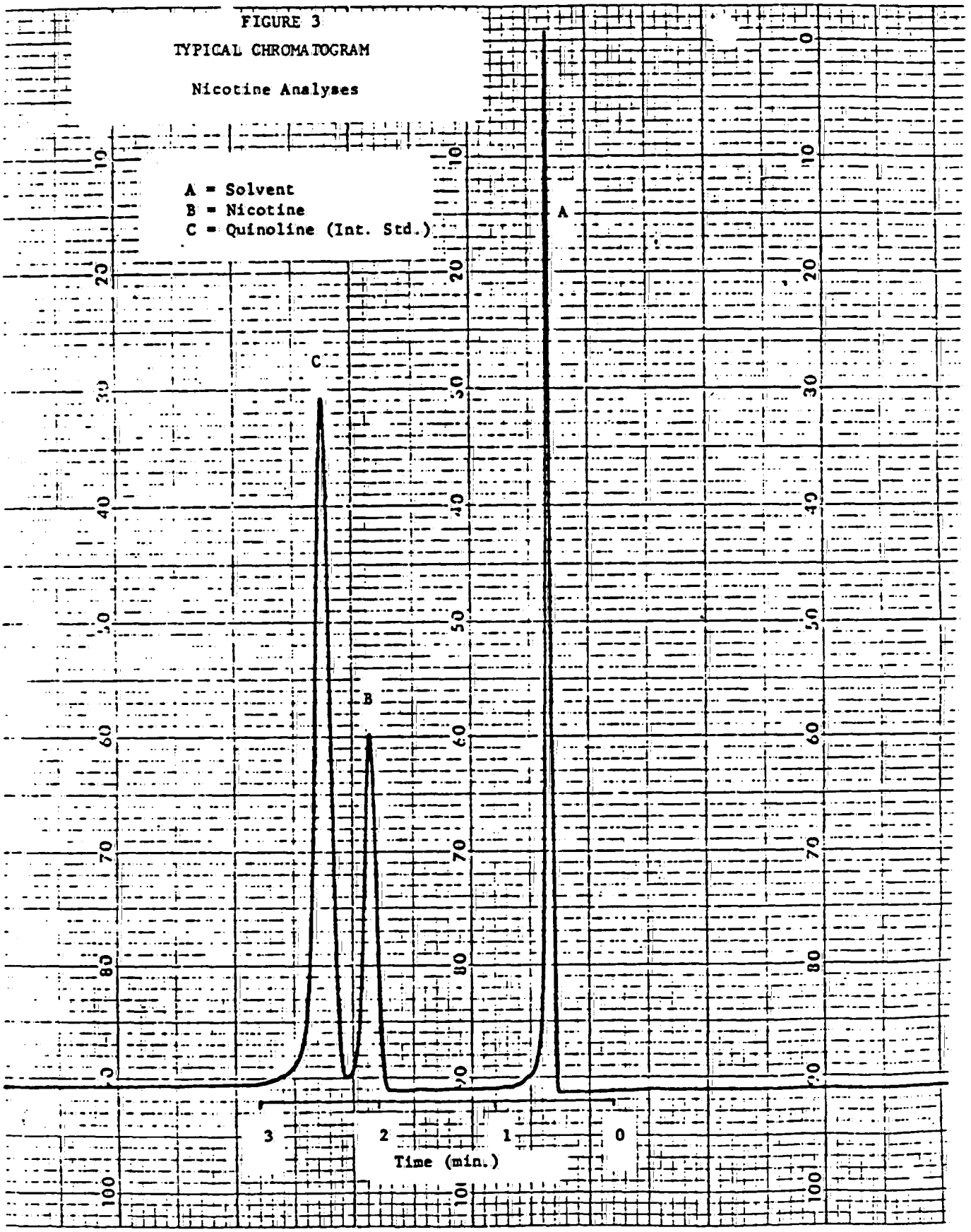
8.11 Steps 8.8 through 8.11 should be ignored if the integrator or computer can perform a multi-point calibration.

8.12 If the gas chromatograph is interfaced with the PAMILA computer system, inject the standards as outlined in Section 12.7.

FIGURE 3

TYPICAL CHROMATOGRAM

Nicotine Analyses



9. Procedure

9.1 Gas Chromatograph Conditions

Column Oven Temperature - 200°C

Injection Size - 2 µl

Thermal Conductivity Detector:

Temperature - 250°C

Sensitivity - 5 (Current = 150 milliamps)

Attenuation - X4

Carrier Gas Flow - 2 mL/min.

Carrier Gas - Helium

Polarity - 'A'

Offset - 'None'

NOTE: Reference and carrier flow should be equal.

Compound Detected - Water

Nitrogen/Phosphorous Detector:

Temperature - 300°C

Air Flow - 50 mL/min.

Hydrogen Flow - 3.2 mL/min.

Collector Voltage - 18-20

Electrometer Range - X100

Attenuation - X1024

Carrier Gas Flow - 15 mL/min.

Carrier Gas - Helium

Compound Detected - Nicotine

9.2 Automatic Sampler Conditions

Operating Mode - Auto

Stop Integrate - 0

Analysis Cycle - 2 min.

Injections per sample - 1

Wash Cycle - Minimum

Syringe Stroke - Stop 1

(Inject - 2 µl)

Total Analysis Time - 3 min.

9.3 Column System Installation

9.3.1 Install a modified H-P 1/8" injection port liner (H-P Part No. 18744A) in the "A" injector. The modification was carried out in the B&W Machine Shop. (See Figure 1.)

9.3.2 Attach the nicotine column to the side outlet of the injector using a 1/8" Graphlok ferrule.

9.3.3 Attach a 1/8" to 1/16" Swagelok reducing union to the outlet of the nicotine column.

9.3.4 Attach a 2' x 0.009" I.D. x 1/16" O.D. stainless steel flow restrictor to the outlet of the nicotine column. This tubing is available from most high-pressure liquid chromatography supply houses.

9.3.5 Connect the outlet of the flow restrictor to the "B" nitrogen/phosphorous detector (N/PD.) using a Supelco reducing ferrule 1/8" to 1/16".

9.3.6 Attach the water column to the bottom outlet of the injector using a 1/8" Graphlok ferrule.

9.3.7 Attach the outlet of the water column to the "A" inlet of the thermal conductivity detector (TCD).

9.3.8 Attach a reference gas line to the "B" side of the TCD. Run a 1/8" copper line from the "B" injector to the TCD. Be sure to first install a H-P injection port liner (H-P Part No. 18744A) in the "B" injector. No injections are made into "B" injector!

9.3.9 This allows the "B" flow controller to be used to adjust the reference gas flow to the TCD.

9.3.10 Install a plug in the inlet of the unused "A" N/PD.

9.3.11 Adjust the carrier gas flow through the water column to 25 mL/min. using the "A" flow controller.

9.3.12 Carefully leak check all connections using a 1:1 solution of methanol:water. DO NOT USE "SNOOP" OR ANY SOAP SOLUTION because they may contain phosphates which could contaminate the N/P detector. If leaks are found, repair and readjust carrier flow.

9.3.13 Allow carrier gas to flow through the column system for 15 minutes before heating to 210°C. Allow the column system to equilibrate ten minutes before making final flow adjustments.

9.3.14 Check the flow through the water column and adjust to 25 mL/min. if necessary.

9.3.15 Adjust TCD reference gas flow to 25 mL/min. using "B" flow controller.

9.3.16 Check carrier flow through the nicotine column. The flow should be 12 mL/min. ±2 mL/min. If the flow falls outside these limits, the length of the flow restrictor must be changed. A shorter restrictor increases the flow and a longer restrictor reduces flow. After the restrictor is changed, the flow through the water column must be adjusted.

9.3.17 Turn-on the thermal conductivity detector. See Section 9.5

9.3.18 Turn-on the nitrogen/phosphorous detector. See Section 9.6.

9.3.19 After the detectors have stabilized, inject 2-3 µL of a smoke sample that contains both internal standards into the "A" injector.

9.3.20 A typical chromatogram should take about three minutes. See Figures 2 and 3.

9.3.21 The temperature of the column system can be adjusted to improve resolution. Increasing the column temperature generally causes faster elution of the compounds.

9.3.22 The carrier gas flow can also be adjusted to improve resolution. Increasing the flow generally causes faster elution of the compounds.

9.3.23 If no response is obtained from the detectors or if the chromatograms do not resemble those in Figures 2 and 3, see Section 9.4 - Troubleshooting.

9.4 Troubleshooting

9.4.1 If a sample is injected and no response is observed from either detector; check for a leaking septum, large column leak at the injector, or a plugged injector. Make sure that the electronic equipment is working properly.

9.4.2 If no response is observed on the TC detector; check for a plugged column, leak at the detector inlet, or detector malfunction.

9.4.3 If no response is observed for the N/P detector; check for a plugged column, plugged restrictor, leak at the detector inlet, collector "BEAD" failure, or other detector malfunction.

9.4.4 Changes in peak retention time usually indicate changes in flow, temperature, or column failure.

9.4.5 "Noise" in the chromatogram usually indicates a gas leak, impure gases, or dirty detector.

9.4.6 Loss of sensitivity with the TC detector usually indicates a leak or dirty filaments (usually accompanied by electronic noise).

9.4.7 Loss of sensitivity for the N/P detector indicates improper collector height, incorrect H_2 or air flow, impure gases, or low collector voltage. Some loss of sensitivity is normal as the collector ages.

9.5 Thermal Conductivity Detector Start-Up

9.5.1 Set the detector temperature to 250°C and allow the temperature to stabilize. This takes two to five hours.

9.5.2 Adjust the flow of the reference side carrier gas using the "B" flow controller to match the flow of the water column.

9.5.3 Set the sensitivity knob at five, the attenuation to four, the offset to none, and the polarity switch to "A."

9.5.4 Inject 2 to 3 µL of the 4 mg water standard. The chromatogram should resemble that shown in Figure 2. A complete chromatogram should take two to three minutes.

9.6 Nitrogen/Phosphorous Detector Start-Up

9.6.1 Set the detector temperature to 300°C and allow temperature to stabilize. This takes one to two hours.

9.6.2 Carrier gas must be flowing through the detector before the collector voltage is turned on or the collector will quickly burn out.

9.6.3 Set hydrogen range valve lever in the down position - 0 to 7 mL/min. (See Figure 4.)

9.6.4 Set hydrogen flow for detector "B" to 3.2 mL/min.

9.6.5 Set air flow for detector "B" to 50 mL/min.

9.6.6 Set meter select switch to "B" detector.

9.6.7 Rotate the voltage knob for detector "B" counterclockwise to stop.

9.6.8 Set the electrometer range to 1.

9.6.9 Set attenuation to X8.

9.6.10 Turn on voltage to the detector "B." The meter will read about ten volts.

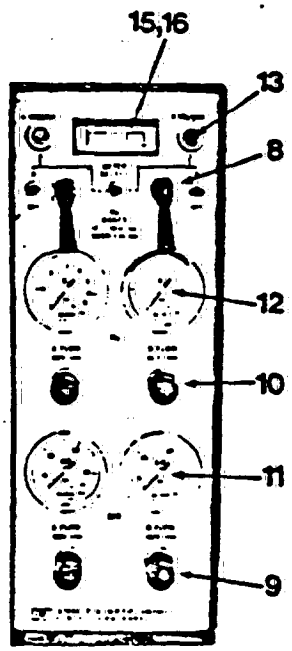
9.6.11 Raise voltage until a 10% rise in the baseline is observed on the recorder.

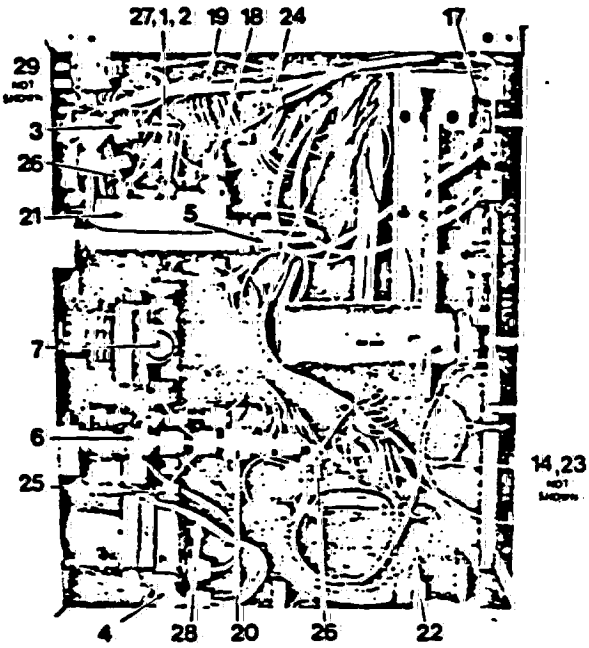
9.6.12 Hold this baseline for one hour, adjusting voltage as necessary to maintain this offset.

9.6.13 After an hour, slowly raise voltage over a period of three hours to 19-20 volts.

FIGURE 4

N-P CONTROL MODULE





Item	Description	Part Number	Qty
1	Front Ferrule, 1/8T	0100-0032	6
2	Back Ferrule, 1/8T	0100-0036	6
3	Tubing Nut, 1/8T	0100-0058	2
4	Male Elbow, 90°, 1/8T, 1/8 PM	0100-0065	2
5	Male Connector, 1/8T, 1/8 PM	0100-0110	6
6	Female Branch Tee, 1/8T, 1/8F, 1/8T	0100-0713	2
7	Pipe Plug, 1/8M NPT, stainless steel	0100-0887	4
8	Toggle Valve	0101-0296	2
9	Pressure Regulator, Air	0101-0352	2
10	Pressure Regulator, H_2	0101-0358	2
11	Pressure Gauge, Air	0101-0360	2
12	Pressure Gauge, H_2	0101-0361	2
13	Round Knob	0370-1091	2
14	Nut, 5/16-20, brass	0590-0385	2
15	Panel Meter, 0-30 VDC	1120-0619	1
16	Meter Support	18789-00120	1

9.6.14 Set the electrometer range to 100.

9.6.15 Set attenuation to X1024.

9.6.16 Using the electrometer coarse zero, set the baseline at 10%.

9.6.17 Inject 2 µL of the highest nicotine standard. The nicotine peak should be 15 to 18 cm high.

9.6.18 If the nicotine peak is off-scale, raise the collector height one division. Repeat the injection and adjust as necessary.

9.6.19 Inject 2 µL aliquot of each nicotine standard.

9.6.20 Determine the peak area ratios of nicotine to internal standard.

9.6.21 On graph paper, plot the standard peak area ratios versus concentration. This must be a straight line.

9.6.22 If non-linear, raise the voltage and repeat. Do not go any higher than 22 or 23 volts - instead replace the collector.

9.6.23 Because the N/P detector can become non-linear as the collector ages, a linearity check (9.6.21) must be performed at the beginning of each day.

9.6.24 When the N/P detector is not going to be used for a long period (over four days), reduce the voltage to about 14 volts.

9.6.25 If the N/P detector is not going to be used for a short while (less than four days), do not change the voltage setting.

9.6.26 Do not turn off the collector voltage except to change septums and columns, etc.

9.7 How to replace the collector in the nitrogen/phosphorous detector (Hewlett-Packard Model 18789A).

9.7.1 Turn off the voltage switches on the control module. (See Figure 4.)

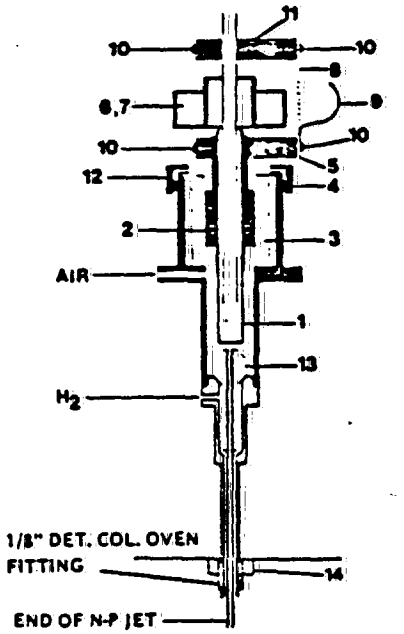
9.7.2 Turn off the hydrogen and air toggle valves on detector.

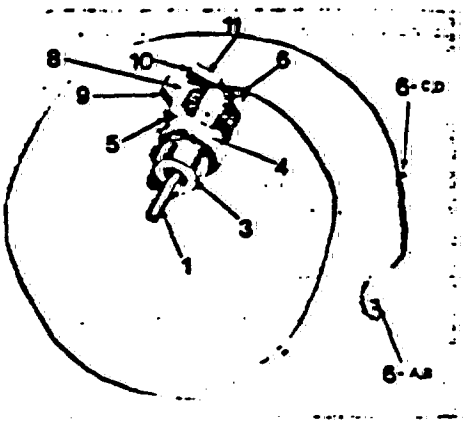
9.7.3 Remove the detector cover by loosening two retaining screws.

9.7.4 Loosen the two collector lock screws. (See Figure 5.)

FIGURE 5

N-P CHIMNEY ASSY.





Item	Description	Part Number	Qty
1	Collector N-P (shown)	18789-60040	*
	Collector, Autoignition (supplied)	18789-60080	*
	Collector, Manual (supplied)	18789-20170	*
2	O-ring	0905-0111	1
3	Insulator	18789-60150	1
4	Knurled Nut (brass)	18789-20110	1
5	Bottom Clamp	18789-20070	1
6	Toroid Assy.	18789-60050	1
A	• Connector	1251-3407	1
B	• Contact	1251-0667	2
C	• Wire Marker A	7120-5376	2
D	• Wire Marker B	7120-5377	2
7	Flat Head Screw, 4-40 x 0.25	2200-0165	2
8	Link Strap	18789-00060	1
9	Contact	18789-20150	1
10	Pan Head Screw, 4-40 x 0.25	2200-0139	4
11	Top Clamp	18789-20060	1
12	Spring (Wave) Washer	2190-0174	1
13	N-P Jet (long-small bore)	18789-20190	*
	N-P Jet (long-large bore)	18789-80060	*
14	Brass Nut, 5/16-20	0590-0385	1

Two (2) each are provided with the detector or installation kit.

9.7.5 Hold the top clamp to one side and pull the collector out with a pair of needle-nose pliers.

9.7.6 Install the replacement collector; push the collector down as far as possible then pull it up three divisions.

9.7.7 Small adjustments can be made to the collector to fine tune the detector when other operating conditions are established.

9.7.8 Tighten the collector lock screws.

9.7.9 Replace the detector cover.

9.7.10 Turn on the hydrogen and air toggle valves on the detector.

9.7.11 Continue as in Section 9.6.2.

9.8 Automatic Sampler Set Up

9.8.1 Install the automatic sampler to inject into the "A" injector (front).

9.8.2 Adjust Stop 1 to inject about 2 µL.

9.8.3 Install a clean syringe in the sampler.

9.8.4 Load the sample vials into the sample cartridge, insert the sample cartridge into the injector and lock in place. Do not put samples in positions 98 and 99.

9.8.5 Start the injection sequence by depressing the run button.

9.8.6 The sampler will start after a time delay.

9.9 Sample Analyses

9.9.1 Cambridge pads are received from the smoke laboratory after they have been transferred to stoppered sample vials.

9.9.2 The sample vials are placed in the "dry-box" where 20 mL of extracting solution is added by means of an auto-pipette. Stopper.

9.9.3 The vials are then shaken for 15 minutes on an up and down shaker.

9.9.4 The vials are removed from the shaker and placed in the "dry box."

9.9.5 Approximately 1 mL aliquots are transferred to the sample vials and capped with a Teflon lined septum cap.

9.9.6 The sample vials are placed in the auto injector sample cartridge.

9.9.7 After every 20 samples, a nicotine control and a water control are analyzed. See Section 11.

10. Calculations

10.1 Determine the areas of the nicotine and water peaks and their appropriate internal standards.

10.2 Calculate the peak area ratios for nicotine to internal standard and for water to internal standard.

10.3 Using the regression equation (determined during standardization), substitute peak area ratios for Y. The value for X will be the amount of nicotine or water.

10.4 Standardization Example

10.4.1 The following peak areas were measured for the water standards:

Standard	Water	Int. Std.
Blank	5,008	49,500
0.5 mg/cig.	10,017	49,648
1.0	16,111	50,513
2.0	25,971	49,479
3.0	36,232	51,217
4.0	45,196	48,921
Blank	4,991	49,427
0.5 mg/cig.	11,131	49,693
1.0	15,917	50,121
2.0	27,119	49,610
3.0	35,013	51.104
4.0	43,991	48,617

10.4.1 The following peak areas were measured for the nicotine standards:

Standard	Nicotine	Int. Std.
Blank	022	97,017
0.10 mg/cig.	5,092	105,094
0.50	24,963	96,958
1.00	45,029	87,111
2.00	105,991	101,091
Blank	012	95,912
0.20	4,897	104,892
0.50	25,103	105,227
1.00	44,971	86,897
2.00	106,171	100,961

10.4.2 The peak area ratios are calculated as follows:

$$\frac{\text{Peak Area (Water or Nicotine)}}{\text{Peak Area (Internal Standard)}} = \text{Peak Area Ratio}$$

10.4.3 The peak area ratios for the standards are as follows:

Standard	Water	Standard	Nicotine
Blank	0.10	Blank	0.00
0.5 mg/cig.	0.20	0.1 mg/cig.	0.05
1.0 mg/cig.	0.32	0.5 mg/cig.	0.26
2.0 mg/cig.	0.52	1.0 mg/cig.	0.52
3.0 mg/cig.	0.71	2.0 mg/cig.	1.05
4.0 mg/cig.	0.92	Blank	0.00
Blank	0.10	0.1 mg/cig.	0.05
0.5 mg/cig.	0.22	0.5 mg/cig.	0.24
1.0 mg/cig.	0.32	1.0 mg/cig.	0.52
2.0 mg/cig.	0.55	2.0 mg/cig.	1.05
3.0 mg/cig.	0.69		
4.0 mg/cig.	0.90		

10.4.4 Perform least squares linear regression on the above data and determine the correlation coefficient, Y-intercept and slope.

	Water	Nicotine
Y-intercept	0.112	-0.005
Slope	0.200	0.526
Corr. Coeff.	0.999	0.999

10.4.5 The standard equations are determined by substituting the above values for the slope and the Y-intercept in the following equation.

$$X = \frac{Y - (Y\text{-Intercept})}{\text{Slope}}$$

where: X = the amount of water or nicotine
Y = peak area ratio of water or nicotine

10.4.6 Thus:

$$\text{Water} = \frac{\text{Peak Area Ratio} - 0.112}{0.200}$$

$$\text{Nicotine} = \frac{\text{Peak Area Ratio} + 0.005}{0.526}$$

10.5 Sample Analyses:

10.5.1 See Section 10.1 and 10.2

10.5.2 Using the equations set up in Section 10.4.6, substitute the peak areas and solve. The results are in mg/cig.

11. Quality Control Procedure

11.1 Nicotine/Water Chemical Control

11.1.1 After every 20 samples, a nicotine and a water control are analyzed.

11.1.2 These controls are the 1 mg nicotine standard and the 1 mg water standard.

11.1.3 Sections 7.3 and 7.4 describe how to make these controls.

11.1.4 The results of the analysis of these controls must agree within specified limits set for this method by the statistician and laboratory supervisor.

11.1.5 If the results fall outside the limits, the method is declared out of control and the analyses must be repeated.

11.1.6 If the results continue to fall outside the limits during repeat analyses, consult Section 12 for remedial action.

11.1.7 If no change in the results are noted after the application of the procedures in Section 12, shut the method down and notify the Manager of Analytical Methods Development.

11.2 Nicotine/Water Smoke Control (Referee)

11.2.1 Two referee samples are smoked per run on a Philip Morris Smoking machine. A "high" level referee - about 12 mg tar with 1 mg nicotine and a "low" level referee with about 5 mg tar with 0.6 nicotine.

11.2.2 These referee samples are analyzed and the nicotine and water results must fall within a specified range determined by the standard deviation of a number of samples analyzed on different days.

11.2.3 If the results are out of range, the Gas Chromatography Laboratory Supervisor should consult with the Smoking Laboratory Supervisor to determine if there was a variance in the smoking run.

11.2.4 If there was a variance during the smoking run, and a repeat analysis of the referee samples shows the results still out of range - the sample must be discarded and the smoking run repeated.

11.2.5 If there was no variance during the smoking run and a repeat analysis still shows the results out of range - consult Section 12 for remedial action.

11.2.6 If following the suggestions in Section 12 results in no significant changes in the results, shut down the method and notify the Manager of Analytical Methods Development.

12. Operational Notes

12.1 The glass liner and septum for "A" injection port should be changed at the beginning of the day. The columns should be at room temperature and the N-P detectors must be turned off during the change.

12.2 The linearity of the N-P detector should be checked at the beginning of each day by injecting each standard in order and plotting the peak area ratios of nicotine to the internal standard versus concentration.

12.3 Good chromatographic practice demands that, when unfamiliar samples are to be analyzed, a preliminary sample containing no internal standard be analyzed to determine if a compound is present that would interfere with the internal standard.

12.4 As the N-P collector ages, the detector response decreases; however, the peak area ratio (nicotine to internal standard) does not change. Changing the attenuation will compensate for this loss. A typical collector can be expected to last from two weeks to three weeks.

12.5 The upper end of the N-P collectors has division marks to aid in adjustment. Each division = 0.025". To adjust the collector, loosen the two lock screws; push collector down as far as possible, then pull it up three divisions. Tighten lock screws.

12.6 We strongly recommend the use of a carrier gas purifier to reduce the amount of oxygen and water in the carrier gas to less than 1 ppm. This will greatly increase column life. We recommend a unit such as the Supelco Carrier Gas Purifier Catalog #2-3800.

12.7 This is the standardization procedure recommended for use with the PAMILA computer system:

12.7.1 After conditioning the column system, standardize using three injections of each: the combined solvent blank - 0.1 mg/cig. nicotine standard solution, and the combined 3.0 mg/ig. water standard -1.5 mg/cig. nicotine standard solution.

12.7.2 After standardization is complete, inject each standard in order to determine response.